Author's response to reviews

Title: Detecting imipenem resistance in Acinetobacter baumannii by automated systems (BD Phoenix, Microscan WalkAway, Vitek 2); high error rates with Microscan WalkAway

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Author's response to reviews: see over
Dear Editor,

We would like to present our revised version of our paper entitled “Comparison of three automated systems (BD Phoenix, Microscan Walkaway and Vitek 2) for detecting imipenem resistance in Acinetobacter baumannii” for your consideration for publication.

We have also read the current “Instructions to Authors” and we will comply with the instructions and stated conditions. All of the named authors agree to the submitted draft of the paper. The content is unpublished and has not been simultaneously submitted to another medical journal.

We would like present our point-by-point description of the changes made as follows.

Major comments

- Difficult to understand why such a high rate of major error, and minor errors when compared to automates with disk diffusion.

As the main focus of the study was imipenem susceptibility, the reference microdilution method was not used for the antibiotics other than imipenem. However, as the reviewer kindly emphasised, there is a high rate of major and minor errors observed between DD and automated systems, for a particular group of antibiotics, the reason of which should be clearly evaluated with further studies including the reference microdilution method. Our comments about the subject is added in the discussion as follows and further studies are addressed:

“It is noteworthy that; inconsistent results with DD and by all automated systems in susceptibilities to antibiotics other than imipenem were also observed in this study; mainly for trimethoprim-sulfamethoxazole, meropenem, cefepime and levofloxacain. Besides when compared to each other; discordances between the three automated systems were also encountered mostly with these antibiotics. Previously high minor error levels were already noted for automated systems when testing β-lactam antimicrobials for Enterobacteriaceae and P. aeruginosa (19, 22, 23). However, the accuracy of the automated systems in susceptibility testing of A. baumannii against the aforementioned antibiotics should be further evaluated with other studies by using the reference BMD method.”
• Lack of description of the resistance mechanism to understand or further interpret discordances between methods
Probable factors for discordances were described an discussed in the modified discussion section

• Discussion need to be rewritten, more focused on obtained results, and explanations for the discrepancies between technics.
Discussion has been rewritten more focused on the obtained results. Sixth, seventh, and eighth paragraphs were added for explanations for the discrepancies for the technics with unacceptable error rates.

Minor comments

• Abstract: No point of such a conclusion. The work is not dedicated to MicroScan, or the authors will have to change their title.
The title has been changed as “Detecting imipenem resistance in *Acinetobacter baumannii* by automated systems (BD Phoenix, Microscan WalkAway, Vitek 2); high error rates with Microscan WalkAway”

• Methods. Identification based on the reference of Bouvet et al in 1989. There should be more recent and molecular approach for identification of Acinetobacter. No sequencing is performed to understand discordances in identification.
Reference for identification has been changed with a recent text book reference. Identification has been confirmed by API 20 NE system with excellent identification confidence levels. We were not able to perform sequencing on the isolates for identification.

• Results: too short, tables are not well introduced in the text and explained in term of presented data. What is the point of Table 2?
Tables are introduced in the text and explained in terms of presented data.
Table 2 was organised in order to give information about the other antibiotic susceptibilities of the whole collection included in the study and take notice to the variations in susceptibility rates against the other antibiotics tested by disk diffusion and three automated sytems as well. Explanations has been added in the result section as:

“Susceptibility rates of the isolates against the tested antibiotics by DD method and the three automated sytems are shown in Table 2. Gentamicine displayed the highest sususceptibility rates, concordantly by all the automated systems, ranging bet ween 58 and 60.4 %. Discordant susceptibility rates were observed mostly in imipenem (20.4% by BD Phoenix, 21.4% by DD, 22.5% by Vitek2 and 46.4% by MicroScan), meropenem (19.4% by BD Phoenix, 19.6% by DD, 32.4% by Vitek2 and 22.3% by MicroScan), cefepime (5.6% by BD Phoenix, 41.1% by DD, 16.2% by Vitek2 and 11.6% by MicroScan) and trimethoprim-sulfamethoxazole (40.7% by BD Phoenix, 29.5% by DD, 49.5% by Vitek2 and 33.9% by MicroScan).

Numbers of discordant results by DD and each automated systems in susceptibility testing to the antibiotics tested are exhibited in Table 3. Discordances were defined as “major” when the isolate was found to be susceptible by one method/system and resistant with the other method/system and “minor” when the isolate was found to be susceptible or resistant by one method/system and intermediate with the other method/system.

For trimethoprim-sulfamethoxazole susceptibilities, high numbers of major discordances were displayed between the DD and the three automated systems; 11, 15 and 23 discordant results were obtained when DD was compared to MicroScan, BD Phoenix and Vitek2, respectively. Major discordances for trimethoprim-sulfamethoxazole were also observed for the automated systems when compared to each other (17 for Vitek2/MicroScan, 13 for Vitek2/Phoenix and 12 for Phoenix/MicroScan). Minor discordances between the three automated systems were mostly observed for meropenem, levofoxacin, cefepime and tetracyclin.”

• **Why 4 strains missing for the Phoenix ?**

Those 4 strains has been described in the result section as “Phoenix unidentified: two strains and misidentified two strains”

• **Why Imipenem is not described in Table 3 ?** In addition, it is difficult to see which
is which.

Imipenem has been added to Table 2.

An explanation has been added to the footnotes of the Table 3 as follows:

“Imipenem was not included in the table; types of errors in susceptibility testing to imipenem by all systems can be derived in detail from Table 1.”

In order to facilitate to see which is which; some of the lines in the table were darkened.

- **Why such high rate of minor errors, for example 44 with cefepim?**

As the main focus of the study was imipenem susceptibility, BMD method was not used for the other antibiotics. However, as the reviewer kindly emphasised, there is a high rate of minor errors observed between DD and automated systems, particularly for a group of antibiotics including cefepim, the reason of which should be evaluated with further studies including the BMD method. Our comments about the subject is added in the discussion as follows and further studies are addressed:

“It is noteworthy that; inconsistent results with DD and by all automated systems in susceptibilities to antibiotics other than imipenem were also observed in this study; mainly for trimethoprim-sulfamethoxazole, meropenem, cefepime and levofloxacin. Besides when compared to each other; discordances between the three automated systems were also encountered mostly with these antibiotics. Previously high minor error levels were already noted for automated systems when testing β-lactam antimicrobials for *Enterobacteriaceae* and *P. aeruginosa* (19, 22, 23). However, the accuracy of the automated systems in susceptibility testing of *A. baumannii* against the aforementioned antibiotics should be further evaluated with other studies by using the reference BMD method.”

- **Difficult to understand why certains technics failed: do the authors have redone experiments with similar inoculum per isolate?** The fact that comparison was performed on 3 sites might indicated that standardisation was difficult to achieve.

In the Methods section “All systems were tested with inocula from the same subculture.” was added and in the Discussion section an explanation has been made as follows:
This study puts forth discordant results between the three widely used automated susceptibility testing methods for testing the imipenem susceptibilities of A. baumannii isolates for consideration. According to the fact that the study was performed in three laboratories; standardisation difficulties might be questioned. However, all systems were tested with inocula from the same subculture and procedures were performed stringly according to the manufacturers recommendations for both inoculum preparation and technical details at every laboratory. All strains, for which the commercial MIC results were discrepant with MIC results from the BMD reference method, were retested by all methods. Besides, the BMD, Etest, DD methods were performed at the same laboratory with the automated system which displayed unacceptable error rates.

Discussion: not sufficiently focused on the results and explanations for the discrepancies.

Discussion has been rewritten focusing on the results and probable explanations for the discrepancies.

Last paragraph p 7 is difficult to understand. How can we propose as a control technic, disk diffusion which presented the highest rate of very major errors

A correction and explanation and has been made as follows in the discussion:

According to our results; DD method produced an unacceptable rate of very major errors (1.8%), slightly higher than the acceptable limit (1.5%), that can be reconsidered accounting the total number of the isolates. In consideration of low cost and requirement of no special equipment; DD method, available in most laboratories, seems to be a useful method for susceptibility testing of A. baumannii to imipenem, however, our data suggest that traditional DD method may result in very low rates of very major errors. Etest method, whereever available, may be used as an accurate testing method to confirm questionable results generated by automated methods and DD for susceptibility testing of A. baumannii to imipenem.”

The sentence in the conclusion “Disk diffusion as a cheap and easy method, and Etest may be used to confirm imipenem susceptibility.” was corrected as “Etest as an easy and reliable method may be used to confirm imipenem susceptibility.”
In the abstract the last sentence has also been changed as “Etest, whereever available, may be used as an easy and reliable method to confirm imipenem susceptibility.”

The following information has also been added to the revised manuscript on editors suggestion:

*** The source of the clinical isolates for this study has been clarified in the Methods section. A sentence was added as “These isolates were selected retrospectively among the strain collection derived from the clinical samples submitted to the microbiology laboratory for routine diagnostic procedures.”

“This research has been performed with the approval of Zonguldak Karaelmas University, Application and Research Hospital Ethics Committee.” has also been added.

*** More detailed description of the statistical analysis of our data has been provided in the Methods section as follows:

“SPSS (ver. 11.5) programme was used for all calculations. Kappa tests was used for agreement. If P values less than 0.05 it was accepted statisicaly significant.

Very major errors were considered when an organism was defined as resistant by the reference method but was categorized as susceptible with the tested system. Major errors were defined when an organism found to be susceptible by the reference method was considered resistant with the system. Minor errors occurred when an organism was considered susceptible or resistant either by the reference microdilution method or with the tested system but intermediate by the other method. An overall category error rate of <10% was considered for an acceptable performance of susceptibility tests, including ≤1.5% of very major errors and ≤3.0% major errors (13).”

We are very grateful for your comments and suggestions on our manuscript. In the hope that this revised paper would meet your expectations and find your interest.

Yours sincerely,

Dr. Canan Kulah, MD